

# Stability of stationary solutions in models of the Calvin cycle

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## Abstract

In this paper results are obtained concerning the number of positive stationary solutions in simple models of the Calvin cycle of photosynthesis and the stability of these solutions. It is proved that there are open sets of parameters in the model of Zhu et. al. [15] for which there exist two positive stationary solutions. There are never more than two isolated positive stationary solutions but under certain explicit special conditions on the parameters there is a whole continuum of positive stationary solutions. It is also shown that in the set of parameter values for which two isolated positive stationary solutions exist there is an open subset where one of the solutions is asymptotically stable and the other is unstable. In related models derived from the work of [4], for which it was known that more than one positive stationary solution exists, it is proved that there are parameter values for which one of these solutions is asymptotically stable and the other unstable. A key technical aspect of the proofs is to exploit the fact that there is a bifurcation where the centre manifold is one-dimensional.

## 1 Introduction

The Calvin cycle is an important part of photosynthesis and many mathematical models have been proposed to describe it [2], [8]. These vary widely in the number of chemical species included and the kinetics chosen for the individual reactions. In what follows we concentrate on some of the simplest models with the aim of obtaining rigorous results on the number of stationary solutions of the models and their stability. It may be hoped that a deeper understanding of the simpler models will lead to new approaches to analysing the more comprehensive ones.

In [15] a model of the Calvin cycle was introduced which is a system of ordinary differential equations describing the concentrations of five substances.

This level of biological detail is similar to that found in the standard textbook [1] on cell biology. Based on computer modelling the authors of [15] conclude that their system has only one steady state for fixed values of the parameters under certain biological restrictions. (A stationary solution of a system of ODE is one which is independent of time and a steady state is an alternative name for a stationary solution.) They explicitly exclude the case of non-isolated steady states from consideration. They do not make general statements about the stability of the steady states although some results of simulations included in the paper indicate the stability of the steady state considered. In what follows we prove that under certain explicit restrictions on the parameters of the system a continuum of positive steady states occurs. We also give a proof that when these restrictions are not satisfied there exist at most two positive steady states for each choice of the parameters. It is shown that there do exist open sets of parameters for which there are two positive steady states. This does not contradict the results of [15] since the biologically motivated fixed choice of Michaelis constants made there excludes the cases where more than one steady state is present in the model.

It is proved that there are parameters for which an asymptotically stable positive steady state exists. Thus for these parameters any solution which has the property that at some time the concentrations are sufficiently close to those in the steady state converges to the steady state at late times. There are also open regions in parameter space for which no positive steady state exists and regions for which the only positive steady state is unstable. The stability of the state where all concentrations are zero is also dependent on the parameters. Thus for some choices of parameters there are solutions for which all concentrations tend to zero at late times and for other choices of parameters no solutions of this kind exist. More detailed versions of these statements can be found in Theorem 1 and Theorem 2 in Sect. 2. It follows from Theorem 4 of [14] that there are runaway solutions of this model where all concentrations tend to infinity at late times.

In [4] the authors introduced what looks at first sight like a small modification of the model of [15] by rescaling two of the coefficients. It turns out, however, that this modifies the dynamics significantly. These authors considered different possibilities for the kinetics. The model of [4] with Michaelis-Menten kinetics is the main subject of Sect. 3. It was already known from [4] and [14] that this model has two positive steady states for certain choices of the parameters. However nothing had been proved about their stability. Here we show that there are parameters for which one of these steady states is stable and one unstable. It is also shown that this implies analogous statements for a more complicated model, also introduced in [4], where each basic reaction is described using a Michaelis-Menten scheme with a substrate, an enzyme and a substrate-enzyme complex. Details are given in Theorem 3.

Sect. 4 is concerned with equations derived from a model introduced in [4] where the concentration of ATP is included as an additional variable and the diffusion of ATP is taken into account. This leads to a system of reaction-diffusion equations. Setting the diffusion coefficient to zero in this model or

restricting consideration to spatially homogeneous solutions gives rise to a system of ODE which was called the MAdh system in [14]. It was proved there that there are parameters for which this model has two positive steady states. However once again nothing was proved about the stability of these solutions. Here we show that for certain values of the parameters one of the steady states is stable and the other unstable. Details are in Theorem 4. The last section contains a summary of the results of the paper and an outlook on possible future developments.

## 2 The model of Zhu et. al.

This section is concerned with a model of the Calvin cycle introduced by Zhu et. al. [15]. The basic system of equations is

$$\frac{dx_{\text{RuBP}}}{dt} = v_5 - v_1, \quad (1)$$

$$\frac{dx_{\text{PGA}}}{dt} = 2v_1 - v_2 - v_6, \quad (2)$$

$$\frac{dx_{\text{DPGA}}}{dt} = v_2 - v_3, \quad (3)$$

$$\frac{dx_{\text{GAP}}}{dt} = v_3 - v_4 - v_7, \quad (4)$$

$$\frac{dx_{\text{Ru5P}}}{dt} = \frac{3}{5}v_4 - v_5. \quad (5)$$

Here  $x_X$  denotes the concentration of the substance X and the substances involved are ribulose biphosphate (RuBP), phosphoglycerate (PGA), diphosphoglycerate (DPGA), glyceraldehyde phosphate (GAP) and ribulose 5-phosphate (Ru5P). The  $v_i$  are reaction rates and are given by the following expressions of Michaelis-Menten type.

$$v_1 = \frac{k_1 x_{\text{RuBP}}}{x_{\text{RuBP}} + K_{m1}}, \quad (6)$$

$$v_2 = \frac{k_2 x_{\text{ATP}} x_{\text{PGA}}}{(x_{\text{PGA}} + K_{m21})(x_{\text{ATP}} + K_{m22})}, \quad (7)$$

$$v_3 = \frac{k_3 x_{\text{DPGA}}}{x_{\text{DPGA}} + K_{m3}}, \quad (8)$$

$$v_4 = \frac{k_4 x_{\text{GAP}}}{x_{\text{GAP}} + K_{m4}}, \quad (9)$$

$$v_5 = \frac{k_5 x_{\text{ATP}} x_{\text{Ru5P}}}{(x_{\text{Ru5P}} + K_{m51})(x_{\text{ATP}} + K_{m52})}, \quad (10)$$

$$v_6 = \frac{k_6 x_{\text{PGA}}}{x_{\text{PGA}} + K_{m6}}, \quad (11)$$

$$v_7 = \frac{k_7 x_{\text{GAP}}}{x_{\text{GAP}} + K_{m7}}. \quad (12)$$

Here the  $k_i$  are the maximal reaction rates and the  $K_{mi}$  Michaelis constants. The concentration of adenosine triphosphate (ATP) is not modelled dynamically but taken to be maintained at a constant value. Notice that, as remarked in [2], the expression for the sixth of these equations given in [15] is not correct and it has been modified accordingly here. While this change affects the biological interpretation of some of the parameters in the equations it does not change the mathematical properties of the model. In [15] the authors looked for positive steady states of this system using a computer program and they concluded that there exists at most one solution of this type for fixed values of the parameters if the concentrations and parameters are in biologically relevant ranges. In what follows we investigate to what extent statements of this type can be proved analytically and what can be said about the stability of the steady states.

Steady states are characterized by the equations

$$v_1 = v_5, 2v_1 = v_2 + v_6, v_2 = v_3, v_3 = v_4 + v_7 \quad \text{and} \quad v_5 = \frac{3}{5}v_4 \quad (13)$$

for the reaction rates. Combining these gives

$$\frac{1}{5}v_4 - v_6 - v_7 = 0. \quad (14)$$

It follows from (13) and (14) that if  $\beta = \frac{v_7}{v_4}$  then

$$\frac{v_6}{v_2} = \frac{1 - 5\beta}{5(1 + \beta)}. \quad (15)$$

For a positive steady state we must have  $0 < \beta < \frac{1}{5}$ . It can be checked that the equations (13) for steady states are equivalent to (14), (15) and the equations

$$v_5 = \frac{3}{5}v_4, v_3 = v_4 + v_7 \quad \text{and} \quad v_1 = \frac{3}{5}v_4. \quad (16)$$

Thus any set  $(v_2, v_4, v_6, v_7)$  which solves (14) and (15) can be completed by means of (16) to a solution of (13). For any solution of (13) there is at most one set of concentrations which give rise to these  $v_i$  and when such concentrations exist they define a steady state of the system (1)-(12). For instance

$$x_{\text{Ru5P}} = \frac{v_5(x_{\text{ATP}} + K_{m52})K_{m51}}{k_5x_{\text{ATP}} - (x_{\text{ATP}} + K_{m52})v_5} \quad (17)$$

provided the denominator is positive and otherwise there is no positive steady state. For convenience we define a parameter  $\kappa = (K_{m7} - K_{m4})(K_{m6} - K_{m21})$ .

**Lemma 1** The system (1)-(12) with given positive parameters satisfying  $\kappa \neq 0$  has at most two positive steady states. If  $\kappa > 0$  or precisely one of the factors in the product defining  $\kappa$  is non-zero it has at most one positive steady state.

**Proof** Suppose first that  $\kappa \neq 0$ . Then both factors are non-zero. Now

$$\frac{v_7}{v_4} = \frac{k_7 x_{\text{GAP}} + K_{m4}}{k_4 x_{\text{GAP}} + K_{m7}}, \quad (18)$$

$$\frac{v_6}{v_2} = \frac{k_6(x_{\text{ATP}} + K_{m22}) x_{\text{PGA}} + K_{m21}}{k_2 x_{\text{ATP}} x_{\text{PGA}} + K_{m6}}. \quad (19)$$

It follows from (18) and  $K_{m7} \neq K_{m4}$  that  $k_7 \neq k_4\beta$  and from (19) and  $K_{m6} \neq K_{m21}$  that  $5k_6(1 + \beta)(x_{\text{ATP}} + K_{m22}) \neq k_2(1 - 5\beta)x_{\text{ATP}}$ . Thus

$$x_{\text{PGA}} = \frac{k_2(1 - 5\beta)K_{m6}x_{\text{ATP}} - 5k_6(1 + \beta)K_{m21}(x_{\text{ATP}} + K_{m22})}{5k_6(1 + \beta)(x_{\text{ATP}} + K_{m22}) - k_2(1 - 5\beta)x_{\text{ATP}}}, \quad (20)$$

$$x_{\text{GAP}} = \frac{k_4\beta K_{m7} - k_7 K_{m4}}{k_7 - k_4\beta}. \quad (21)$$

The expressions for  $x_{\text{PGA}}$  and  $x_{\text{GAP}}$  can be substituted back into the expressions for the reaction rates  $v_4$  and  $v_6$  to get

$$v_4 = \frac{k_4\beta K_{m7} - k_7 K_{m4}}{\beta(K_{m7} - K_{m4})}, \quad (22)$$

$$v_6 = \frac{k_2(1 - 5\beta)K_{m6}x_{\text{ATP}} - 5k_6(1 + \beta)K_{m21}(x_{\text{ATP}} + K_{m22})}{5(1 + \beta)(x_{\text{ATP}} + K_{m22})(K_{m6} - K_{m21})}. \quad (23)$$

As a consequence of (14) we need that  $v_6 = (\frac{1}{5} - \beta)v_4$  in order to get a steady state. This implies the vanishing of the cubic polynomial  $p(\beta)$  given by

$$(1 - 5\beta)(k_4\beta K_{m7} - k_7 K_{m4})[(1 + \beta)(x_{\text{ATP}} + K_{m22})(K_{m6} - K_{m21})] - [k_2(1 - 5\beta)K_{m6}x_{\text{ATP}} - 5k_6(1 + \beta)K_{m21}(x_{\text{ATP}} + K_{m22})]\beta(K_{m7} - K_{m4}). \quad (24)$$

The sign of  $K_{m6} - K_{m21}$  is the same as that of  $k_2(1 - 5\beta)K_{m6}x_{\text{ATP}} - 5k_6(1 + \beta)K_{m21}(x_{\text{ATP}} + K_{m22})$  since  $v_6 > 0$ . This is in turn the same as the sign of  $5k_6(1 + \beta)(x_{\text{ATP}} + K_{m22}) - k_2(1 - 5\beta)x_{\text{ATP}}$  since  $x_{\text{PGA}} > 0$ . The sign of  $K_{m7} - K_{m4}$  is the same as that of  $k_4\beta K_{m7} - k_7 K_{m4}$  since  $v_4 > 0$ . This is in turn the same as the sign of  $k_7 - k_4\beta$  since  $x_{\text{GAP}} > 0$ .

The sign of the coefficient of the leading term in the polynomial  $p$  is opposite to that of  $K_{m6} - K_{m21}$ . The sign of  $p(0)$  is also opposite to that of  $K_{m6} - K_{m21}$ . The sign of  $p(1/5)$  is that of  $K_{m7} - K_{m4}$ . Under the assumptions of Lemma 1 the sign of  $p(0)$  is opposite to that of  $p$  for large negative values of its argument. Hence  $p$  has at least one negative root and at most two positive ones. This gives the first conclusion of the lemma. If the signs of  $K_{m7} - K_{m4}$  and  $K_{m6} - K_{m21}$  are the same then  $p$  changes sign in the interval  $(0, 1/5)$  and again for  $\beta > 1/5$ . Thus it has precisely one root in the interval  $(0, 1/5)$ . Now consider the case that  $K_{m7} - K_{m4} = 0$ . Then  $k_7 - k_4\beta = 0$  and  $\beta$  is determined by the reaction constants. Under the assumptions of Lemma 1 the inequality  $K_{m6} - K_{m21} \neq 0$  holds in this case and so (20) holds. Thus  $x_{\text{PGA}}$ ,  $v_2$  and  $v_6$  are determined. The equation  $v_6 = (\frac{1}{5} - \beta)v_4$  then determines  $v_4$ . This means that all reaction rates and all concentrations are determined. The proof in the case where  $K_{m7} - K_{m4} \neq 0$  and  $K_{m6} - K_{m21} = 0$  is similar.

**Lemma 2** Consider the system (1)-(12) with parameters satisfying  $\kappa < 0$ . Then there are choices of the remaining parameters for which there exist precisely two steady states and choices for which there do not exist any steady states. If, on the other hand,  $\kappa > 0$  there are choices of the remaining parameters for which there exists precisely one steady state.

**Proof** Consider the case  $\kappa < 0$ . Suppose first that  $K_{m7} - K_{m4} > 0$  and consider  $p(\beta)$  for some fixed value of  $\beta \in (0, 1/5)$ . Fix a value of  $\frac{k_7}{k_4}$  and a choice of all parameters except  $k_4$  and  $k_7$ . Under the given assumptions the expression in the second line of (24) is fixed. The expression in the first line is a fixed negative constant times  $k_4(\beta K_{m7} - (k_7/k_4)K_{m4})$ . It follows directly from the definitions of  $v_4$  and  $v_7$  that under the assumptions of the lemma the latter expression is always positive. Thus the expression in the first line is negative and can be made arbitrarily large in magnitude by choosing  $k_4$  large. Hence for large enough values of  $k_4$  it can be concluded that  $p(\beta) < 0$  and it follows from the intermediate value theorem that  $p$  has two roots in the interval  $(0, 1/5)$ . Starting from one of these values of  $\beta$  it is possible to define  $x_{PGA}$  and  $x_{GAP}$  by means of (20) and (21). It has already been shown that the denominator of the right hand side of (21) does not vanish and it follows in a similar way from the definitions of  $v_2$  and  $v_6$  that the denominator of the right hand side of (20) does not vanish. This provides corresponding values of  $v_2$ ,  $v_4$ ,  $v_6$  and  $v_7$ . By construction they satisfy (14) and (15). Hence they can be completed to a solution of (13). If  $k_1$ ,  $k_3$  and  $k_5$  are chosen sufficiently large the reaction rates can be reproduced by suitable values of  $x_{RuBP}$ ,  $x_{DPGA}$  and  $x_{Ru5P}$ . Thus a steady state is obtained and this gives the first conclusion of the lemma. The inequality  $\beta > \frac{k_7 K_{m4}}{k_4 K_{m7}}$  ensures that  $\beta$  is bounded away from zero for all steady states. This implies a fixed positive lower bound for the expression in the second line of (24). Hence this expression dominates the expression in the first line for  $k_4$  small and the sum is positive, contradicting the existence of a root of  $p$ . This gives the second conclusion of the lemma. The proofs in the case where  $K_{m6} - K_{m21} > 0$  are similar, with the role played by  $k_4$  and  $k_7$  in the preceding argument being taken over by  $k_2$  and  $k_6$ .

Now consider the case  $\kappa > 0$ . It was shown in the proof of Lemma 1 that in this case  $p$  has precisely one root in the interval  $(0, 1/5)$ . The rest of the proof that there exists a steady state for suitable choices of the other parameters is then as in the case where there are two roots.

**Lemma 3** Consider the system (1)-(12) with parameters satisfying  $K_{m7} - K_{m4} = K_{m6} - K_{m21} = 0$ . If

$$\frac{k_6(x_{ATP} + K_{m22})}{k_2 x_{ATP}} = \frac{1 - 5k_7/k_4}{5(1 + k_7/k_4)} \quad (25)$$

then the steady states form a one-dimensional continuum. If (25) does not hold then there are no positive steady states.

**Proof** When  $K_{m7} - K_{m4}$  and  $K_{m6} - K_{m21}$  are zero it follows as in the proof of Lemma 1 that  $\frac{v_7}{v_4} = \frac{k_7}{k_4}$  and  $\frac{v_6}{v_2} = \frac{k_6(x_{ATP} + K_{m22})}{k_2 x_{ATP}}$ . The second part of the lemma then follows from (15). When (25) holds we can proceed as follows. Choose  $v_4$  to be an arbitrary number less than  $k_4$ . Then define  $v_7 = \frac{k_7}{k_4} v_4$ . Next let  $v_6 = (\frac{1}{5} - \beta) v_4$  and define  $v_2$  by the relation  $v_6 = \frac{k_6(x_{ATP} + K_{m22})}{k_2 x_{ATP}} v_2$ . These quantities can be completed to a solution of (13) and this in turn gives rise to a steady state of the evolution equations provided  $k_1$ ,  $k_3$  and  $k_5$  are sufficiently large.

The main results which have been proved are summed up in the following theorem, which is a consequence of Lemma 1 - Lemma 3.

**Theorem 1** Consider the system of [15] with all parameters positive.

- (i) There exist at most two isolated positive steady states.
- (ii) If there exist two isolated positive steady states then  $(K_{m7} - K_{m4})(K_{m6} - K_{m21}) < 0$  and if this inequality is satisfied then there is an open region in the space of the other parameters for which two positive steady states exist.
- (iii) If  $(K_{m7} - K_{m4})(K_{m6} - K_{m21}) > 0$  there is an open region in the space of the other parameters for which precisely one steady state exists.
- (iv) There are values of the parameters where the quantities  $K_{m7} - K_{m4}$  and  $K_{m6} - K_{m21}$  are zero for which there exists a one-dimensional family of steady states. If either of these two quantities is non-zero then a continuum of steady states is not possible.

**Proof** (i) follows from Lemma 1 and Lemma 3. (ii) follows from Lemma 1 and Lemma 2. (iii) follows from Lemma 2. (iv) follows from Lemma 1 and Lemma 3.

It is instructive to compare the results of this theorem with the assertions made in [15]. There fixed values are assumed for the Michaelis constants and this implies that  $K_{m7} - K_{m4} = 4.5$  and  $K_{m6} - K_{m21} = 0.51$ , putting us in the region where Theorem 1 implies that there exists at most one positive steady state.

Note that in general if  $L_1 = x_{\text{RuBP}} + \frac{1}{2}x_{\text{PGA}} + \frac{3}{5}x_{\text{DPGA}} + \frac{3}{5}x_{\text{GAP}} + x_{\text{Ru5P}}$  then

$$\frac{dL_1}{dt} = -\frac{1}{2} \left( v_6 - \frac{1}{5}v_2 \right) - \frac{3}{5}v_7. \quad (26)$$

This is a Lyapunov function for  $5v_6 \geq v_2$ . A sufficient condition for this inequality to hold is that  $K_{m6} \leq K_{m21}$  and  $5k_6 \geq \frac{k_2 x_{\text{ATP}}}{x_{\text{ATP}} + K_{m22}}$ . It can be concluded that when the parameters satisfy these inequalities there are no positive steady states and other types of behaviour such as periodic solutions can also be ruled out.

Next the stability of the steady states will be examined. The determinant of the derivative of the right hand side of the system (1)-(5) is the sum of the product of the diagonal elements and the product of the off-diagonal elements. Thus it is a positive factor times

$$-5 \frac{\partial(v_2 + v_6)}{\partial x_{\text{PGA}}} \frac{\partial(v_4 + v_7)}{\partial x_{\text{GAP}}} + 6 \frac{\partial v_2}{\partial x_{\text{PGA}}} \frac{\partial v_4}{\partial x_{\text{GAP}}}. \quad (27)$$

This expression can be manipulated further using the relation  $\frac{\partial v_4}{\partial x_{\text{PGA}}} = \frac{K_{m4} v_4^2}{k_4 x_{\text{PGA}}^2}$  and the analogous expressions for the derivatives of  $v_2$ ,  $v_6$  and  $v_7$ . This leads to the following form of (27)

$$\begin{aligned} & \frac{1}{x_{\text{PGA}}^2 x_{\text{GAP}}^2} \left[ -5 \left( \frac{K_{m21} v_2^2 (x_{\text{ATP}} + K_{m22})}{k_2 x_{\text{ATP}}} + \frac{K_{m6} v_6^2}{k_6} \right) \left( \frac{K_{m4} v_4^2}{k_4} + \frac{K_{m7} v_7^2}{k_7} \right) \right. \\ & \left. + 6 \frac{K_{m21} v_2^2 (x_{\text{ATP}} + K_{m22}) K_{m4} v_4^2}{k_2 k_4 x_{\text{ATP}}} \right]. \end{aligned} \quad (28)$$

Dividing by the positive factor  $v_2^2 v_4^2$  and multiplying by  $k_4 x_{\text{PGA}}^2 x_{\text{GAP}}^2$  leads to the expression

$$\begin{aligned}
& -5 \left( \frac{K_{m21}(x_{\text{ATP}} + K_{m22})}{k_2 x_{\text{ATP}}} + \frac{K_{m6}(1 - 5\beta)^2}{25k_6(1 + \beta)^2} \right) \left( K_{m4} + \frac{k_4 K_{m7} \beta^2}{k_7} \right) \\
& + 6 \frac{K_{m21} K_{m4} (x_{\text{ATP}} + K_{m22})}{k_2 x_{\text{ATP}}}.
\end{aligned} \tag{29}$$

Without examining it in any detail we see that this expression has at most four zeroes for given values of the parameters and that in particular they are isolated.

All the coefficients in the characteristic polynomial other than the determinant are positive and it is an increasing function for non-negative values of its argument whose derivative at zero is positive. Thus either the linearization has precisely one positive eigenvalue or there is none and when there is a zero eigenvalue it is of multiplicity one. In general it is hard to determine the sign which distinguishes these two cases. Consider the case discussed in Lemma 2 where decreasing the value of  $k_4$  takes us from a situation with two positive steady states to one with no positive steady states. There is a first value  $\gamma$  of  $k_4$  for which there are no longer two steady states and at that point there must be exactly one. At the parameter value  $\gamma$  the linearization of the right hand side of the equations must have a non-trivial kernel. Thus in that case the characteristic polynomial has a root at zero and this root is simple. Under suitable restrictions on the parameters all the other eigenvalues have negative real parts so that, in particular, there are no eigenvalues which are purely imaginary and different from zero. This will be proved later. When this holds the centre manifold at that point is one-dimensional. (Some relevant background on centre manifold theory can be found in the Appendix.) The value of  $\beta$  corresponding to this steady state must be a zero of (29). If  $k_4$  is increased slightly there are two steady states. If (27) held for one of these then it would have to have the same value of  $\beta$  as the solution for  $k_4 = \gamma$ . This can be true for at most one of the two solutions under consideration and so at least one of these has no zero eigenvalue. Consider now the perturbed centre manifold in the sense of the Appendix corresponding to a value of  $k_4$  close to the critical value where there are two steady states. Let us fix an orientation of this one-dimensional manifold so that we can call one direction along it left and the other right. The sign of the vector field to the left of both steady states is the same as its sign to the right of both steady states. The vector field along this manifold changes sign at at least one of its two zeroes (where the relevant eigenvalue is non-zero) and hence also at the other. It follows that on the perturbed centre manifold one of the steady states is a sink and the other a source. If it could be proved that all the eigenvalues corresponding to eigenvectors transverse to the centre manifold have negative real parts then we would have shown that one of the steady states is asymptotically stable. In the other situation discussed in Lemma 2, where there is precisely one positive steady state, another approach can be used to obtain information about stability. In that case we consider the limit  $k_6 \rightarrow 0$ . In the limit (27) simplifies and shows that the sign of the critical quantity determining



the sign of an eigenvalue of the linearization at the positive steady state is the same as that of  $5k_7K_{m4} - k_4K_{m7}$ . It follows by continuity that for  $k_6$  small both signs occur for different values of the parameters.

Now the remaining four eigenvalues will be considered. Start with a steady state and corresponding reaction rates  $v_i$ . Now modify  $k_3$  in such a way that it approaches  $v_3$  from below. For each such value of  $k_3$  the given value of  $v_3$  can be produced by a unique value of  $x_{\text{DPGA}}$  and in this way we get a one-parameter family of steady states. As  $k_3$  approaches  $v_3$  the concentration  $x_{\text{DPGA}}$  tends to infinity and the derivative  $\frac{\partial v_3}{\partial x_{\text{DPGA}}}$  tends to zero. The other elements of the linearization remain unchanged. Thus in the limit the matrix tends to one with four negative eigenvalues and one eigenvalue zero. It follows that for values of  $k_3$  close enough to the limit there are four eigenvalues with negative real parts. Information has now been obtained on the stability properties of some positive steady states. Next we consider the stability properties of the solution at the origin, which exists for all values of the parameters. In this case (27) becomes

$$-5 \left( \frac{K_{m2}(x_{\text{ATP}} + K_{m22})}{k_2 x_{\text{ATP}}} + \frac{K_{m6}}{k_6} \right) \left( \frac{K_{m4}}{k_4} + \frac{K_{m7}}{k_7} \right) + 6 \frac{K_{m2}(x_{\text{ATP}} + K_{m22})}{k_2 x_{\text{ATP}}} \frac{K_{m4}}{k_4}. \quad (30)$$

It is again helpful to look at the limit  $k_6 \rightarrow 0$ . Then the critical quantity determining the sign of an eigenvalue becomes  $k_4K_{m7} - 5k_7K_{m4}$ . The sign of the real parts of the four remaining eigenvalues can be controlled in the limit  $k_3 \rightarrow 0$ . These observations are summed up in the following theorem.

**Theorem 2** Consider the system of [15] with all parameters positive.

- (i) There is an open set of parameter values for which there exist one asymptotically stable and one unstable positive steady state.
- (ii) There is an open set of parameter values for which the unique positive steady state is asymptotically stable and an open set for which the unique positive steady state is unstable.
- (iii) There is an open set of parameter values for which the origin is asymptotically stable and an open set for which it is unstable.

### 3 The Michaelis-Menten model of Grimbs et. al.

In [4] the authors introduced a variant of the model of [15] where the stoichiometric coefficients are rescaled so as to make them all integers. The equations are

$$\frac{dx_{\text{RuBP}}}{dt} = v_5 - v_1, \quad (31)$$

$$\frac{dx_{\text{PGA}}}{dt} = 2v_1 - v_2 - v_6, \quad (32)$$

$$\frac{dx_{\text{DPGA}}}{dt} = v_2 - v_3, \quad (33)$$

$$\frac{dx_{\text{GAP}}}{dt} = v_3 - 5v_4 - v_7, \quad (34)$$

$$\frac{dx_{\text{Ru5P}}}{dt} = 3v_4 - v_5. \quad (35)$$

Different models were considered in [4] with different kinetics. In one of these, which is studied in this section, Michaelis-Menten kinetics is used. Although it is not stated explicitly in [4] we assume that the model is identical to that of [15] except for the modified stoichiometric coefficients. In other words, the  $v_i$  are defined as in equations (6)-(12) in the previous section except that  $x_{\text{GAP}}$  is replaced by  $x_{\text{GAP}}^5$  in the expression for  $v_4$ . For short we call this the MM model. In another model discussed in [4] each of the basic reactions is replaced by a Michaelis-Menten scheme with substrate, enzyme and substrate-enzyme complex and the elementary reactions are given mass action kinetics. Following [4] we call this the MM-MA model (Michaelis-Menten via mass action). As mentioned in [14] steady states of the MM model are in one-to-one correspondence with steady states of the MM-MA model with fixed total amounts of substrates and enzymes. It was shown in [14] that for suitable values of the parameters the MM-MA model has more than one positive steady state with the same total amounts of substrates and enzymes. It follows that for suitable values of the parameters the MM model has more than one positive steady state. In [14] no information was obtained on the stability of the steady states. In what follows it will be shown that there are parameter values for which one of the positive steady states is stable and the other is unstable.

We now investigate steady states of the MM system directly. There is a calculation for the reaction rates analogous to that in the last section. For a steady state  $v_1 = v_5$ ,  $2v_1 = v_2 + v_6$ ,  $v_2 = v_3$ ,  $v_3 = 5v_4 + v_7$  and  $v_5 = 3v_4$ . Combining these gives

$$0 = v_3 - 5v_4 - v_7 = v_2 - 5v_4 - v_7 = 2v_1 - 5v_4 - v_6 - v_7 = v_4 - v_6 - v_7. \quad (36)$$

It follows that if  $\beta = \frac{v_7}{v_4}$  then

$$\frac{v_6}{v_2} = \frac{1 - \beta}{5 + \beta} \quad (37)$$

For a positive steady state we must have  $0 < \beta < 1$ . It can be checked that the equations for steady states are equivalent to (36), (37) and the equations

$$v_5 = 3v_4, v_3 = 5v_4 + v_7, v_1 = 3v_4. \quad (38)$$

Thus we see that as in the last section solutions of (36) and (37) can be completed to steady states of the whole system. An analogous statement holds in the limiting case  $k_6 = 0$ , where  $v_6 = 0$ ,  $v_4 = v_7$  and  $\beta = 1$ . Then the equations (38) must be complemented by the equation  $v_2 = v_3$ .

In the case of the MM system the route to analysing steady states used in the previous section does not appear useful. It is possible to express  $\frac{v_7}{v_4}$  as a function of  $x_{\text{GAP}}$  but this relation cannot be solved for  $x_{\text{GAP}}$ . For this reason

we now concentrate on the limiting case  $k_6 = 0$  where we have  $v_4 = v_7$ . Some information on the case  $k_6 \neq 0$  will be obtained later. In the case  $k_6 = 0$  the main equation to be solved is

$$\frac{k_4 x_{\text{GAP}}^4}{K_{m4} + x_{\text{GAP}}^5} = \frac{k_7}{K_{m7} + x_{\text{GAP}}}. \quad (39)$$

Rearranging gives

$$(k_4 - k_7)x_{\text{GAP}}^5 + k_4 K_{m7} x_{\text{GAP}}^4 = k_7 K_{m4}. \quad (40)$$

It turns out (cf. [14], Lemma 2) that this equation has two positive solutions precisely when  $k_4 < k_7$  and  $\frac{1}{5}k_4 K_{m7} \left[ \frac{4k_4 K_{m7}}{5(k_7 - k_4)} \right]^4 < k_7 K_{m4}$ . There is a bifurcation when  $k_7 - k_4 = \frac{1}{5} \left[ \frac{(\frac{1}{5}k_4 K_{m7})^5}{k_7 K_{m4}} \right]^{\frac{1}{4}}$ . This happens exactly when the derivatives of the terms on both sides of (39) are equal. Once (39) has been solved for  $x_{\text{GAP}}$  this can be completed to a steady state as explained above.

When  $k_6 \neq 0$  things are more complicated. One tractable special case is that where  $K_{m21} = K_{m6}$ . Then we get

$$v_4 \left[ 1 - 6 \left( \frac{k_6}{k_2 + k_6} \right) \right] = v_7. \quad (41)$$

Provided  $k_2 > 5k_6$  the analysis of this relation is just as in the case  $k_6 = 0$  except for the fact that  $k_4$  is replaced by  $\left[ 1 - 6 \left( \frac{k_6}{k_2 + k_6} \right) \right] k_4$ . Once  $x_{\text{GAP}}$  has been determined for a steady state it is possible to reconstruct the concentrations of Ru5P, RuBP, PGA and DPGA.

Starting from the result in the case  $k_6 = 0$  we can obtain a result for  $k_6$  small but non-zero using the implicit function theorem. The reaction rates satisfy the equations  $v_4 = v_6 + v_7$  and  $\frac{v_6}{v_2} = \frac{1 - \frac{v_7}{v_4}}{5 + \frac{v_4}{v_7}}$ . Substituting the definitions of the reaction rates into these gives two equations for the concentrations  $x_{\text{PGA}}$  and  $x_{\text{GAP}}$ . When  $k_6 = 0$  the first of these equations simplifies to the equation for  $x_{\text{GAP}}$  alone which has just been analysed. When it has two solutions the derivative of  $v_4 - v_6 - v_7$  with respect to  $x_{\text{GAP}}$  is non-zero at each of these. To show that these solutions persist for  $k_6$  small using the implicit function theorem it suffices to show that the partial derivative of  $\frac{v_6}{v_2}$  with respect to  $x_{\text{PGA}}$  is non-zero for  $k_6 = 0$ . This is easily checked. Since it was shown in [14] that there are parameter values for which there exists a continuum of solutions of the MM-MA system with the same values of the conserved quantities there must also exist parameters for which the MM system has a continuum of solutions but solutions of this type will not be studied further here.

Next the stability of the steady states will be investigated by looking at the derivative of the right hand side of the equations. Consider first the case  $k_6 = 0$ . The constant term in the characteristic polynomial is the product of a positive quantity with

$$\frac{5k_4 K_{m4} x_{\text{GAP}}^4}{(K_{m4} + x_{\text{GAP}}^5)^2} - \frac{k_7 K_{m7}}{(K_{m7} + x_{\text{GAP}})^2} \quad (42)$$

This vanishes precisely when the bifurcation condition holds. The characteristic polynomial has properties analogous to those we saw in the last section. When the characteristic polynomial vanishes at zero it has a simple root there. Its derivative at that point is non-zero. There are no other positive real roots. It will be shown later that there are parameter values for which there exist no eigenvalues which are purely imaginary but different from zero. When this holds the centre manifold at the bifurcation point is one-dimensional and we have a situation similar to that in the last section. When there are two steady states on the perturbed centre manifold both of them are hyperbolic. When  $k_6$  is perturbed a little away from zero these two solutions continue to exist and to be hyperbolic. That it can be arranged that four of the eigenvalues have non-zero (in fact negative) real parts can be shown by the same method as in the previous section, considering the limit  $k_3 \rightarrow 0$ . It can be seen that one of the steady states is a hyperbolic sink. The unstable manifold of the other steady state coincides with part of the perturbed centre manifold and is a heteroclinic orbit connecting the two steady states.

In [14] it was shown how a parameter  $\epsilon$  can be introduced into the MM-MA system so as to obtain the MM system formally in the limit  $\epsilon \rightarrow 0$ . This was done for a more general class of systems including the MM-MA system for the Calvin cycle as a special case. In fact this is more than a formal limit and rigorous results on stability can be obtained using geometric singular perturbation theory (GSPT). (A basic reference for this subject is [3] and a summary of some of the key ideas can be found in the Appendix of [6].) To achieve this it is necessary to show that certain eigenvalues, the transverse eigenvalues in the sense of GSPT, have non-zero real parts. In the example considered here we will show that they all have negative real parts. Under these circumstances we can say the following. If there is a hyperbolic steady state of the MM system,  $k$  of whose eigenvalues have negative real parts then for  $\epsilon$  small the corresponding steady state of the MM-MA system is hyperbolic and has  $k+t$  eigenvalues with negative real parts, where  $t$  is the number of transverse eigenvalues. In particular, if the solution of the MM system is stable the same is true for the solution of the MM-MA system.

In the general notation used in [14] the substrates are denoted by  $A_i$  and in the present example they are the five substances occurring in the MM model. The enzymes catalysing the seven reactions are denoted by  $E_\alpha$ . The complex formed by the binding of  $A_i$  to  $E_\alpha$  is denoted by  $A_iE_\alpha$ . After a suitable rescaling the MM-MA system takes the form

$$\dot{x} = f(x, y, \epsilon), \quad (43)$$

$$\epsilon \dot{y} = g(x, y, \epsilon), \quad (44)$$

which is the standard form used in GSPT. In the example  $x$  consists of the variables  $x_{A_i}$ ,  $y$  consists of the variables  $x_{A_iE_\alpha}$  and the variables  $x_{E_\alpha}$  have been eliminated using the conservation laws for the total amounts of enzymes. The transverse eigenvalues are the eigenvalues of the derivative of the right hand side of the equation for  $y$  with respect to the variable  $y$ . In this type of system

the evolution equations for the different substrate-enzyme complexes are all decoupled from each other since each enzyme only binds to one substrate. Hence the matrix whose eigenvalues are to be calculated is diagonal. The eigenvalues can be read off from the equations in [14]. For example in the notation of that paper the eigenvalue corresponding to the variable  $x_{\text{GAPE}_4}$  is  $-k_{10}x_{\text{GAPE}_4}^5 - k_{11} - k_{12}$ . It can be concluded that in the case  $k_6 = 0$  there exist parameter values for which the MM-MA system has two hyperbolic steady states, one of which is asymptotically stable and the other of which has a one-dimensional unstable manifold. By continuity the same holds for  $k_6$  small and non-zero. Moreover the stable and unstable steady states are connected by a heteroclinic orbit.

**Theorem 3** There are positive parameter values for the MM system for which there exist one stable and one unstable positive steady state. The same holds for the MM-MA system with suitable fixed values of the total amounts of substrates and enzymes.

## 4 The MAdh model

A model for the Calvin cycle including diffusion was introduced in [4]. It uses mass action kinetics. Restricting consideration to spatially homogeneous solutions or setting the diffusion constant to zero leads to a system of six ordinary differential equations which was studied in [14] and was called the MAdh model. It was shown that for certain values of the parameters there exist two positive steady states. The stability of those solutions was not determined. Here we will show how information about their stability can be obtained. In the MAdh model equations (31)-(35) hold and are supplemented by the equation

$$\frac{dx_{\text{ATP}}}{dt} = -v_2 - v_5 + v_8. \quad (45)$$

The reaction rates are

$$v_1 = k_1 x_{\text{RuBP}}, \quad (46)$$

$$v_2 = k_2 x_{\text{PGA}} x_{\text{ATP}}, \quad (47)$$

$$v_3 = k_3 x_{\text{DPGA}}, \quad (48)$$

$$v_4 = k_4 x_{\text{GAP}}^5, \quad (49)$$

$$v_5 = k_5 x_{\text{Ru5P}} x_{\text{ATP}}, \quad (50)$$

$$v_6 = k_6 x_{\text{PGA}}, \quad (51)$$

$$v_7 = k_7 x_{\text{GAP}}, \quad (52)$$

$$v_8 = k_8 (c - x_{\text{ATP}}) \quad (53)$$

with a positive constant  $c$ . The equations for the  $v_i$  with  $1 \leq i \leq 7$  satisfied by steady states of the MM system are also valid for the MAdh system. There is an extra reaction rate  $v_8$  for the regeneration of ATP and an extra equation  $v_8 = v_2 + v_5$  which can be solved at the end if required.

In [14] it was shown that positive steady states of the MAdh model are only possible if  $x_{\text{ATP}} > \frac{5k_6}{k_2}$  and that determining them is equivalent to finding solutions of the following system of two equations for the concentrations  $x_{\text{GAP}}$  and  $x_{\text{ATP}}$ .

$$f_1(x_{\text{GAP}}, x_{\text{ATP}}) = k_4(k_2x_{\text{ATP}} - 5k_6)x_{\text{GAP}}^4 - k_7(k_2x_{\text{ATP}} + k_6) = 0, \quad (54)$$

$$f_2(x_{\text{GAP}}, x_{\text{ATP}}) = x_{\text{ATP}} - c + \frac{8k_4}{k_8}x_{\text{GAP}}^5 + \frac{k_7}{k_8}x_{\text{GAP}} = 0. \quad (55)$$

A solution of these equations can be completed to a steady state of the whole system by defining

$$x_{\text{RuBP}} = \frac{3k_4}{k_1}x_{\text{GAP}}^5, \quad (56)$$

$$x_{\text{PGA}} = \frac{2k_1x_{\text{RuBP}}}{k_2x_{\text{ATP}} + k_6}, \quad (57)$$

$$x_{\text{DPGA}} = \frac{k_7x_{\text{GAP}}}{k_3} + \frac{5k_4x_{\text{GAP}}^5}{k_3}, \quad (58)$$

$$x_{\text{Ru5P}} = \frac{3k_4x_{\text{GAP}}^5}{k_5x_{\text{ATP}}}. \quad (59)$$

Moreover, depending on the parameters the number of solutions of the equations (54) and (55) is zero, one or two. We call the values of the parameters for which there is exactly one solution the bifurcation values. They are precisely the points where the Jacobian determinant of the mapping  $(f_1, f_2)$  vanishes. It is clear that the zero sets of  $f_1$  and  $f_2$  are smooth curves. A bifurcation point occurs precisely when these two curves are tangent to each other.

We claim that the characteristic polynomial has a zero eigenvalue at the bifurcation point. This is because as  $c$  is varied while the other parameters are fixed two steady states coalesce at the bifurcation point. We will show later that there are parameter values for which this zero has multiplicity one. To do this we study the linearization of the right hand side of the equations. To compute its eigenvalues it is necessary to calculate the determinant of a certain matrix. Adding suitable multiples of the second and fifth columns of this matrix to the last column simplifies the matrix while leaving its determinant unchanged. The determinant of the linearization at a steady state is a positive multiple of

$$\begin{aligned} & k_8 \left[ \left( 1 + \frac{k_6}{k_2x_{\text{ATP}}} \right) (25k_4x_{\text{GAP}}^4 + k_7) - 30k_4x_{\text{GAP}}^4 \right] \\ & + \frac{6k_4k_6x_{\text{GAP}}^5}{x_{\text{ATP}}(k_2x_{\text{ATP}} + k_6)} (40k_4x_{\text{GAP}}^4 + k_7). \end{aligned} \quad (60)$$

Denote this function of  $x_{\text{GAP}}$  and  $x_{\text{ATP}}$  by  $f_3$ . Note that it does not depend on  $c$ . Its partial derivative with respect to  $x_{\text{ATP}}$  is everywhere negative. Thus the zero set of  $f_3$  is a smooth curve. For parameter values corresponding to a bifurcation point the zero sets of  $f_1$  and  $f_2$  are tangent to each other and the

zero set of  $f_3$  must pass through their point of tangency. If  $c$  is increased the intersection point splits into two and these two points lie in the zero set of  $f_1$ . We would like to show that neither of these two points can lie in the zero set of  $f_3$  if the parameters are sufficiently close to their bifurcation value. For in that case if the zero eigenvalue at the bifurcation point is of multiplicity one the linearization has no zero eigenvalues after bifurcation and the two steady states are hyperbolic. To get this conclusion it suffices to show that in a neighbourhood of the bifurcation point the zero sets of  $f_1$  and  $f_3$  intersect in only one point. The equation  $f_1 = 0$  can be used to solve for  $x_{\text{ATP}}$ . Substituting this into the expression for the determinant and multiplying by a suitable positive quantity gives a polynomial equation for the value of  $x_{\text{GAP}}$  at an intersection of the zero sets of  $f_1$  and  $f_3$ . We know that this polynomial vanishes at the bifurcation point and provided it does not vanish identically its zero set is discrete, which gives the desired result. That the polynomial does not vanish identically follows from the fact under the condition  $f_1 = 0$  the expression (60) diverges as  $x_{\text{GAP}}$  tends to infinity. For in that situation  $x_{\text{ATP}}$  tends to a constant value and the second term in (60) dominates the first.

To show that there are parameter values for which the zero eigenvalue at the bifurcation point does indeed have multiplicity one we look at the limit where  $k_5$  tends to zero while the other parameters are held constant. The linearization then tends to a limit which is simpler than it is in general. In the limit it can be easily seen that there are eigenvalues zero,  $-k_7 - 25k_4x_{\text{GAP}}^4$ ,  $-k_3$  and  $-k_1$ . It remains to study the determinant of a  $2 \times 2$  matrix. These are the roots of the quadratic polynomial

$$\lambda^2 + (k_2x_{\text{ATP}} + k_6 + k_8 + k_2x_{\text{PGA}})\lambda + (k_2x_{\text{ATP}} + k_6)k_8 + k_2k_6x_{\text{PGA}}. \quad (61)$$

They have negative real parts. Thus when  $k_5$  is close to but not equal to zero the bifurcation point is such that all eigenvalues except one have negative real parts. The sign of the remaining eigenvalue is then the same as that of the determinant. In this situation the stable steady state is that at which the concentration of ATP is higher. Putting these facts together leads to the following result.

**Theorem 4** There exist positive parameter values for the MAdh system for which there exist one stable and one unstable positive steady state.

## 5 Summary and outlook

There are a number of things which have been proved about the dynamics of simple models of the Calvin cycle where the unknowns are the concentrations of five sugar phosphates. In the present paper and the previous work on which it builds information has been obtained on the number and stability of positive steady states under various assumptions as well as solutions where the concentrations tend to zero or infinity at late times. Similar information has been obtained on the MM-MA model and the MAdh model described in the paper. It is known that  $\omega$ -limit points for which some concentrations are zero must be such that the concentrations of all sugar phosphates vanish [14]. All these

models except the MM-MA and MAdh models are cooperative systems. This means that all the off-diagonal elements of the derivative of the right hand side of the equations are non-negative. In addition the derivative is irreducible, i.e. it leaves no linear subspace defined by the vanishing of a subset of the variables invariant. Thus, by a theorem of Hirsch [7], in the set of initial data giving rise to bounded solutions all but those belonging to a set of measure zero converge to the set of steady states at late times. When the steady states are isolated this means that each of these solutions converges to a steady state. On the other hand it is not known whether every bounded solution converges to a steady state and it is also not known whether there exist periodic solutions. Furthermore, the maximum number of isolated steady states for a given model is generally not known. For the model of [15] and the MAdh model there are never more than two of these. For the MM model this question is still open and it is not clear how it could be approached.

There are many other models of the Calvin cycle and in general they include many chemical species. An interesting model was introduced in [11] and a modified version of it was studied in [12] and [13]. In these models the kinetics is more complicated than mass action or Michaelis-Menten with the concentrations of some species modulating the rate of reactions where they are not among the reactants. These models and simplified variants of them with mass action kinetics have been investigated mathematically in [10]. Information was obtained about solutions for which some concentrations tend to zero at late times. This is related to the biological phenomenon known as overload breakdown. It is also related to the cases in the present paper where concentrations tend to zero at late times. There remains much to be understood concerning the dynamics of these models. It would be desirable to understand the mathematical relation of these models to the models including less chemical species.

The techniques used in this paper might also be applied to other problems. Here it was seen that when the centre manifold at the bifurcation is of dimension one it may be possible to obtain results about stability on the basis of quite limited information. This may be compared with the example in [6] of a model of a biochemical system where the method used to control a bifurcation with a one-dimensional centre manifold made use of a lot more detailed calculations. Another technique which played a central role was looking at limiting values of the parameters to get information about what types of stability properties can occur. In many arguments it turned out to be useful to carry the calculations as far as possible in terms of the reaction rates before using the dependence of these rates on the concentrations.

## Appendix: some background on centre manifolds

Here some facts about centre manifolds which are relevant to the paper are reviewed briefly. Consider a system of ODE of the form  $\dot{x} = f(x)$  and a steady state  $x_0$ . In other words  $f(x_0) = 0$ . In general the derivative  $Df(x_0)$  has eigenvalues with positive, zero and negative real parts. The corresponding gen-



eralized eigenvectors define three linear subspaces  $E_u$ ,  $E_c$  and  $E_s$  whose direct sum is the whole space.  $E_c$  is the centre subspace. There exists a manifold  $V_c$  (in general non-unique) called the centre manifold of  $x_0$ , which contains  $x_0$ , is invariant under the evolution defined by the ODE and whose tangent space at  $x_0$  is  $E_c$ . The dynamics close to  $x_0$  is topologically equivalent to a product of two factors. One factor is the dynamics on any centre manifold of  $x_0$ . The other is topologically equivalent to a linear system with  $E_u$  and  $E_s$  of the same dimensions as in the original system. This means that if we understand the qualitative properties of solutions which are close to  $x_0$  and lie on the centre manifold we obtain information on the dynamics of all solutions close to  $x_0$ . All steady states sufficiently close to  $x_0$  lie on the centre manifold of  $x_0$ . For more information on these matters we refer to [9], in particular Chapter 5 of that book.

Next suppose that we have instead of a single ODE a family  $\dot{x} = f(x, \lambda)$  of ODE depending on a parameter  $\lambda$  and suppose that  $(x_0, 0)$  is a steady state. An extended system with one more dimension can be defined by adjoining the equation  $\dot{\lambda} = 0$ . The centre manifold of the extended system at  $(x_0, 0)$  has one more dimension than that of the centre manifold of the original system at  $x_0$ . Since  $\lambda$  is time independent the extended centre manifold is foliated by invariant manifolds of constant  $\lambda$  which agree with the original centre manifold for  $\lambda = 0$ . Let us call a manifold of this type a perturbed centre manifold. This construction is useful for the study of the case where  $(x_0, 0)$  is a bifurcation point, i.e. the centre manifold for the system with  $\lambda = 0$  at  $x_0$  has dimension greater than zero. In this paper we are concerned with the case that the dimension of the centre manifold is one so that the dynamics on these invariant manifolds is of dimension one. For  $(x, \lambda)$  sufficiently close to  $(x_0, 0)$  all steady states of the system for a fixed value of  $\lambda$  lie on the perturbed centre manifold corresponding to that value of the parameter.

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